# MLN51, l'un des composants coeur du complexe de jonction des exons (EJC), est essentiel à la formation des granules de stress.

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La protéine MLN51 appartient à un complexe multiprotéique nommé complexe de jonction des exons (EJC). L'EJC est déposé au cours de l'épissage en amont de la jonction exonique et influence le devenir cytoplasmique des ARN messagers en favorisant l'export nucléaire, la traduction et la dégradation. Récemment, les protéines Magoh, Y14, eIF4AIII et MLN51 sont apparues comme étant les composants du cœur de l'EJC. Ces quatre protéines sont capables de faire la navette entre le noyau et le cytoplasme. Néanmoins, à la différence des autres composants, qui sont majoritairement nucléaire, MLN51 est plutôt retrouvée dans le cytoplasme, suggérant un rôle supplémentaire de MLN51 dans le cytoplasme.

La protéine MLN51 possède une architecture complexe. Sa moitié amino-terminale contient une région conservée nécessaire à l'incorporation de la protéine dans l'EJC. Cette région, appelée SELOR pour Speckles Localizer and RNA binding module, intervient dans la liaison de MLN51 à l'ARN, interagit avec Magoh et permet l'adressage de la protéine en périphérie de régions sub-nucléaires nommées speckles. La partie carboxy-terminale est riche en proline.

Nous avons montré qu'au cours d'un stress cellulaire, la protéine MLN51 était recrutée dans des agrégats cytoplasmiques appelés granules de stress. MLN51 s'associe spécifiquement aux granules de stress *via* sa région carboxy-terminale. Cette région, ne contenant pas le domaine SELOR, n'intervient pas dans l'association de MLN51 à l'EJC, indiquant que le recrutement de MLN51 dans les granules de stress s'effectue indépendamment de l'EJC.

La surexpression de MLN51 n'induit pas la formation des granules de stress mais par contre son inhibition ou la surexpression d'un mutant MLN51, dont de la région carboxy-terminale a été supprimée, abolit complètement la formation des granules de stress. Ces données suggèrent que MLN51 est essentielle à la formation des granules de stress.

# Hyperphosphorylation induces Src-dependent FAK translocation from focal adhesion to membrane ruffle

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Tyrosine phosphorylation of focal adhesion kinase (FAK) is involved in the disassembly process of focal adhesion by mechanisms that involved the time-residency of FAK at these sites. Here, we analyze in living human astrocytoma cells, how phosphorylation of FAK regulates its trafficking at focal adhesions. Upon pervanadate-induced FAK hyperphosphorylation, focal adhesions started to disassemble while phosphorylated FAK appeared highly expressed at membrane ruffles. To analyze the dynamic relationship between FAK trafficking and phosphorylation events, we used a new approach for monitoring directly tyrosine phosphorylation. The recently described Src reporter was able to monitor phosphotyrosine proteins. Its intensity was linearly correlated with phospho-FAK and therefore, could be used as a phospho-FAK reporter in living cells. Time-lapse movie revealed that upon addition of pervanadate, phospho-FAK decreases at focal adhesions while in the meantime, it starts to appear at newly-formed membrane ruffles. Kinetics analysis shows that lost of phospho-FAK at focal adhesion was time-correlated with the appearance of membrane ruffles-containing phospho-FAK. This effect was abolished in presence of the specific Src inhibitor PP2. Our findings demonstrate that upon hyperphosphorylation FAK is translocated from focal adhesions to membrane ruffles, via a Src-dependent mechanism.

# Ikaros is a physiological repressor of Notch target genes

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The Notch signalling pathway plays a major role in biology. It regulates cell fate during embryonic development and hematopoiesis, and homeostasis of various types of stem cells. Thus, activation of this pathway must be tightly controlled, as its deregulation can promote tumorigenesis. The transcriptional mechanisms that repress Notch-dependent activation
remain poorly understood. We study the role of the Ikaros transcription factor in silencing Notch activity in T lymphocytes. During T cell differentiation, Notch activity is required at early stages, and becomes repressed at later stages. Our data show that Ikaros-deficient mice (called IkL/L mice) develop T cell leukemias, which are always associated with an early activation of the Notch pathway. In addition, Ikaros strongly represses the expression of several Notch target genes when overexpressed in cell lines. The molecular basis for this expression may lie in the similarity of the sequences recognized by Ikaros and RBP-Jk, the transcription factor downstream of Notch. Ikaros and RBP-Jk both bind sequences that contain the same central motif TGGGAA, and we have shown that in the promoter of the Hes-1 gene, both factors compete to repress and activate transcription, respectively. We propose that Ikaros competes with RBP-Jk to repress a set of common target genes and that this mechnism plays a central role in silencing Notch targets in T cells during differentiation.

# An inhibitor of HIV-1 integrase suppresses the nucleic acid chaperone function of the HIV-1 nucleocapsid protein

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To decrease the probability of the appearance of resistant HIV-1 mutants during chemotherapy, it is necessary to combine several drugs preferably having different targets. However, by increasing the number of anti-viral chemicals, one increases the number of possible adverse side effects. Therefore, a molecule that simultaneously impairs more than one target of HIV-1 would be very promising for the development of novel anti-HIV agents. In this view, a 11nt deoxyribonucleotide conjugated with 9-aminoacridine (Acr-GT) attracted our attention because it was expected to be active against two proteins of HIV-1. First, Acr-GT was shown to dissociate the DNA/HIV-1 integrase complex (Pinskaya et al., 2004). Second, G- and T- (or U-) -rich single-stranded oligonucleotides are known to bind tightly HIV-1 nucleocapsid protein (NC) (Vuillemier et al., 1999). NC is a promising target for anti-HIV drugs, because this protein has a highly conserved sequence and is essential for several key events in the HIV life cycle. For instance, NC acts as a chaperone protein during reverse transcription by directing the two obligatory strand transfers necessary for the synthesis of a complete proviral DNA. In the present work, we studied the RNA and DNA forms of the 11nt sequence, as well as their modifications (conjugates with 9-aminoacridine, with eosin and the 2'O-methylated oligonucleotide). We examined the binding of these oligonucleotides to NC and their effects on two NC-chaperoned processes underlying the first strand transfer. It is found that due to their affinity to NC, the undecanucleotides efficiently inhibit the NC-promoted destabilization of the cTAR stem-loop and its hybridization with the complementary TAR sequence of HIV genome. 2'O-methylation increases the inhibition activity, while coupling with 9-aminoacridine or eosin does not affect the inhibition. The 2'O-methylated and eosin conjugated derivative showed strong inhibitory activity in cellular assays, presumably by targeting both the integrase and NC. Our results open new prospects for the development of anti-HIV agents.

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# Tracking of HPV-tumour specific retargeted adenoviral vectors

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Second cause of mortality per cancer for woman worldwide, cervical cancer is mostly caused by infection with high-risk group of HPV. An innovative and complementary approach to the conventional treatment is the use of gene therapy. Most adenoviruses used for gene therapy have been based on serotype 5. Unfortunately, its primary cell receptor, the coxsackie-adenovirus receptor (CAR), is widely express. Consequently, various strategies have been developed to abolish this natural tropism and to retarget adenovirus to tumour cells. We present here the construction of genetically modified adenoviruses with fiber gene carrying two mutations: (i) one to abolish the interaction with its natural primary receptor: the KO1 mutation (ii) the other consist in insertion of different coding sequences for HPV tumour targeting peptides that we previously isolated by phage display. After virus production, we assessed essential steps of gene transfer to HPV-positives cells including binding and internalisation by the use of virions covalently conjugated to fluorophores.

We demonstrate that genetically modified adenoviruses are able to enter specifically HPV-positive cells and that they have lost their natural tropism for non-HPV cells. We also show that retargeted adenoviruses do not colocalise with wild type virus suggesting another intracellular trafficking pathway leading to nuclear transfer. To improve the specificity of the treatment for clinical carcinoma cells, advantage will be taken of retargeted adenovirus to attempt a specific delivery of suicide genes into HPV-positive cells.

# Direct Vpr-Vpr interaction monitored by Fluorescent Life Time Imaging (FLIM)

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Vpr is a 96 amino acid protein with an N- terminal part required for virion incorporation, nuclear localization and Vpr oligomerisation. The C-terminal part is involved in the G2 cell cycle arrest, apoptosis and for the interaction with NCp7 and nucleic acid. The structure of Vpr fragments and of full length Vpr was obtained by NMR with addition of solvent or with micelles. The difficulties to obtain structural elements could be explained by the potency of Vpr to oligomerise through formation of leucine zippers. In order to characterize the Vpr oligomerisation in a cellular context a series of experiments using GFP and mCherry chimeric proteins were achieved using fluorescence lifetime imaging microscopy (FLIM) with two photon microscopy as a reliable tool to detect protein-protein contact. From energy transfer measurements, Vpr-Vpr interaction is shown in HeLa cells at the nuclear envelop level but also in the cytoplasm and the nucleus. This energy transfer depends on the position of the fluorescent protein on the N or C terminus in Vpr. Deletion or substitution of amino acid predicatively involved in the Vpr tridimensional folding elicits a large decrease of energy transfer while mutation of other residues does not hamper Vpr oligomerisation.

# Suppression of cervical carcinoma cell growth by intracytoplasmic co-delivery of anti-oncoprotein E6 antibody and siRNA.

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Human papillomaviruses (HPV) are epitheliotropic viruses implicated in the etiology of a number of human cancers, notably the cancer of uterine cervix. More than 99% of invasive cervical carcinomas harbor high risk HPV, such as HPV16 and HPV18. HPV infection is associated with decreased apoptosis and increased cell proliferation. These activities have been related to the oncogenic potential of the early viral proteins E6 and E7, which disrupt the control of cell proliferation by interacting with p53 and pRb, respectively. It has been shown, silencing of E6 gene expression in HPV-positive cell lines by transfection of siRNA with cationic lipids restores the dormant p53 tumor suppressor pathway. Since cationic lipids can also be used for intracytoplasmic delivery of proteins (called transduction), we tested whether the delivery of monoclonal antibodies that specifically bind to HPV16 E6 and neutralize its biological activity *in vitro* could restore p53 function in tumor cells. Here, we demonstrate that the 4C6 antibody is efficiently delivered into the cell cytoplasm using a lipidic reagent used for siRNA transfection. The transduction of 4C6 resulted in the accumulation of p53 protein in nuclei of CaSki and SiHa cells (HPV 16-positive cells), but not in HeLa cells (HPV 18 positive cells). Furthermore, addition of a dimer of peptide corresponding to the 4C6 epitope to the transduction mixture allowed an increase of the antibody intracytoplasmic delivery and a concomitant increase of the nuclear accumulation of p53 protein. With this system, the proliferation of CaSki and SiHa cells was strongly diminished. This was correlate with an increase level of p21, a p53 responsive gene. However, no apoptosis was detectable. Cell growth was almost totally suppressed when E6-specific siRNA to the transduction complex. The results indicate that the activity of E6 oncoprotein can be down-regulated *in vivo* by lipid-mediated antibody delivery and that antibodies and siRNA act synergistically when co-delivered. This novel and easily-implemented targeting strategy might be of therapeutic value.

# Reduced expression of vascular connexins (Cx37, Cx40 and Cx43) and calcium-activated potassium channels ($SK_{Ca}$ and $IK_{Ca}$) in mesenteric arteries of angiotensin II-treated rats

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*Objective:* EDHF (endothelium-derived hyperpolarizing factor)-mediated responses are associated with an hyperpolarization of vascular smooth muscle cells. The main objective of the present study were (1) to determine the role of endothelial calcium-activated potassium channels ($SK_{Ca}$ and $IK_{Ca}$) and connexins Cx37, Cx40, Cx43, which compose myoendothelial gap junctions, in EDHF-mediated responses in isolated rat mesenteric artery and (2) to examine whether the expression of these components is altered in hypertensive rats.

*Methods:* Male Wistar rats were treated with angiotensinII (AngII, 0.4mg/kg/day) during 21 days using osmotic-pumps. Arterial pressure was measured by tail-cuff plethysmography. Reactivity of isolated mesenteric arteries was measured in organ chambers and EDHF-mediated component of relaxation was examined in presence of $N^{w}$-nitro-L-arginine and indomethacin. The expression level of connexins (Cx37, Cx40, Cx43), $IK_{Ca}$ and $SK_{Ca}$ was quantified by immunohistochemistry and RT-PCR, in segments of mesenteric arteries from 2 groups.

*Results*: In mesenteric arteries from control rats, 18α-GA and carbenoxolone, gap junction blockers, abolished EDHF-mediated relaxation to acetylcholine. The combination of charybdotoxin or Tram-34 + apamin, blockers of $IK_{Ca}$ and $SK_{Ca}$, abolished these responses. The chronic administration of AngII increased the blood pressure. In isolated mesenteric arteries from AngII-treated rats, EDHF-mediated responses to acetylcholine were impaired in comparison to control rats whereas global and NO-mediated relaxations were unaltered. The expression of the three connexins, $SK_{Ca}$ and $IK_{Ca}$ were decreased in mesenteric arteries from AngII-treated rats.

*Conclusion:* These findings indicate that the three connexins, $SK_{Ca}$ and $IK_{Ca}$ play a major role in EDHF-mediated responses in the rat mesenteric artery. The abolition of these responses in AngII-treated rats could be attributed to a decreased expression of Cx37, Cx40, Cx43, $SK_{Ca}$ and $IK_{Ca}$ channels.

# In search for molecular probes to explore central roles of vasopressin receptors

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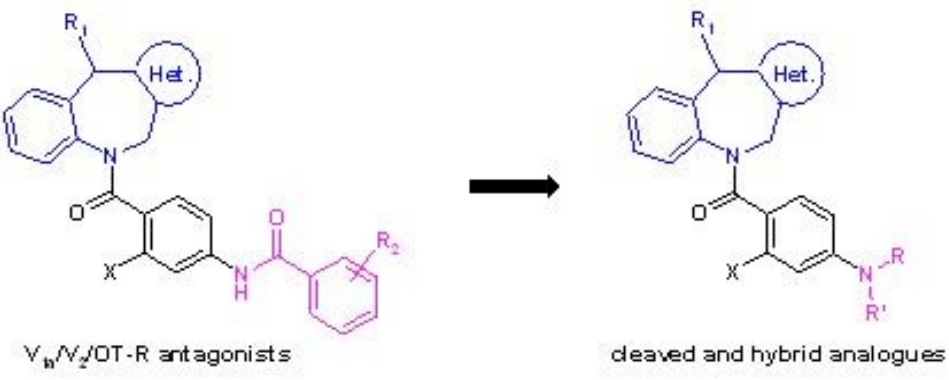
Neuropeptide hormones arginine-vasopressin (AVP) and oxytocin (OT) have recently been described to play a crucial role in a broad range of attachment behaviours including social interactions, pair bonding, sexual behaviours or maternal care[1], all important for species survival. This mainly involves two of their four receptors, namely the $V_{1A}$ and OT receptors, which are coupled to G proteins. Designing specific and bioavailable agonists to explore these functions *in vivo* remains a challenging task. Therefore, we developped a rational approach based on the docking of a series of non peptide, selective or non selective antagonists[2] into the models of $V_{1A}$, $V_2$ and OT receptors. Their binding mode was compared to the AVP and OT binding one. We then designed and synthesised cleaved and hybrid analogues to investigate the switch in selectivity from one subtype to another and the switch in efficacy from antagonism to agonism.




Results indicate that the affinity, specificity and efficacy molecular discriminants are very subtle both on receptors and on their ligands despite the high homology of receptor subtypes and the high similarity of their ligands.

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# Synthèse et fonctionnalisation de 2,3-benzodiazépines : Vers la construction d'une chimiothèque focalisée.

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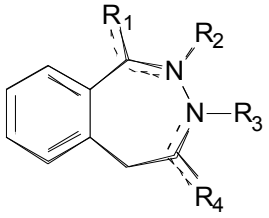
Les travaux menés au sein de notre laboratoire sur les pyridazine-3,6-diones et phtalazine-1,4-diones, composés connus pour leurs potentiels pharmacologiques (Alzheimer, Inflammation,…) nous ont amenés à développer de nouvelles méthodologies chimio- et/ou régiosélectives de fonctionnalisation de ces hétérocycles à 6 chaînons.

Dans notre quête de nouvelles molécules bioactives, nous nous sommes intéressés à l'homologue supérieur (hétérocycle à 7 chaînons) des phtalazines, les 2,3-benzodiazépines, système bien moins documenté que les 1,4-benzodiazépines et pourtant tout aussi intéressant d'un point de vue pharmacologique.




$R_1 = R_4 =$ O, Ar, Amine
$R_2 = R_3 =$ Alkyl, Ns

Notre objectif a consisté à développer de nouvelles méthodologies permettant la conception d'une chimiothèque focalisée autour du noyau 2,3-benzodiazépine. L'introduction de groupements Aryls, Alkyls ou Amines en position 1 à 4 permet de combiner autour du noyau benzodiazépine diverses fonctions capable de générer des interactions (hydrophobes, ioniques, électrostatiques,…) avec d'éventuelles cibles biologiques (Enzymes, RCPG, …).

# Lrh-1 is required for the establishment of the neuronal network of the developing spinal cord and dorsal root ganglia

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Liver Receptor Homolog-1 (LRH-1, NR5A2) is a nuclear receptor involved in the regulation of metabolism and proliferation that composes together with SF-1 (Steroidogenic Factor-1, NR5A1) the NR5A subfamily of nuclear receptors. In this study we describe a new and unexpected function of LRH-1 in the central nervous system. Using different specific markers of the neuronal populations, we have shown that LRH-1 is expressed in a sub-population of the ventral interneurons V0, V1, V2, V3 in the spinal cord and in the proprioceptive neurons of the dorsal root ganglia (DRG). The specific deletion of LRH-1 in differentiated neurons, using Synapsin-Cre mouse model, leads to defects in the sensory motor functions consequently to a loss of proprioceptive neurons and afferent projections. More precisely, the number of proprioceptive neurons expressing the ETS gene ER81 decreases in LRH-1 mutant mice. LRH-1 deletion in differentiated neurons also affects the expression of transcription factors regulating neuronal fate during the development of the ventral spinal cord such as En1, Evx1 and Nkx2.2. Moreover, the LRH-1 deletion leads to an increased expression of members of the ephrin and cadherin family which function as guidance and adhesion molecules. In combination, our data suggests a role for of LRH-1 in the formation of axons or synaptic connections in the ventral spinal cord and dorsal root ganglia and indicate that LRH-1 is involved in the control of locomotor function.

# Role of mouse frataxin in the biosynthesis of mitochondrial and extra-mitochondrial iron-sulfur clusters.

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Friedreich ataxia, a neurodegenerative disease associating cardiomyopathy, is caused by severely reduced levels of the mitochondrial protein frataxin. We have previously generated conditional mouse models which reproduce important pathophysiological and biochemical features of the human disease: progressive cardiac hypertrophy, progressive sensory and cerebellar ataxia, multiple mitochondrial Fe-S enzyme deficiency, time-dependent intramitochondrial iron accumulation. The mitochondrial Fe-S deficit precedes the iron accumulation, indicating that in mammalian cells, frataxin may play a major role in the mitochondrial Fe-S biosynthesis as previously suggested in yeast.

In mammalian cells, the involvement of mitochondria in the biosynthesis of extra-mitochondrial Fe-S clusters remains controversial since an independent cytosolic Fe-S biosynthesis machinery has been suggested. In the present work, we assessed the activity of three extra-mitochondrial containing enzymes in frataxin-deleted mouse models: Nth1, a nuclear DNA repair enzyme involved in the base excision repair of oxidized bases; Xanthine oxido-reductase (XOR), a cytosolic enzyme of the purine catabolism pathway; and Glutamine phosphoribosylpyrophosphate amidotransferase (GPAT), a cytosolic enzyme of the purine biosynthesis pathway. All enzymes were clearly affected in mutant mice compared to wild type mice. The mitochondrial localization of frataxin was also confirmed, excluding the presence of a cytosolic pool of frataxin in adult mouse tissues. Interestingly, the mitochondrial scaffold IscU, which is involved in the assembly of Fe-S clusters from elemental iron and sulfur, was progressively diminished in mutant mice. All these results suggest that, in mammalian cells, mitochondria are involved in the biosynthesis of extra-mitochondrial Fe-S proteins, and that the impairment of Fe-S biosynthesis induced by the frataxin deletion might be triggered by the decrease of the IscU protein level.

# Rôle de la Parp-2 dans le contrôle épigénétique de la différenciation cellulaire.

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La Poly(ADP-ribose)polymérase 2 (Parp-2) représente avec la Parp-1, un facteur essentiel du système de réparation par excision de base ce qui définit son rôle clé dans la surveillance et la protection du génome [1].

Lors d'une recherche de partenaires spécifiques de Parp-2 dans les cellules de testicules par une approche de spectrométrie de masse, nous avons isolé le régulateur de la transcription TIF1ß (Facteur de Transcription Intermédiaire 1ß), co-répresseur universel de la grande famille des facteurs de transcription à domaine KRAB et doigt de zinc. TIF1ß interagit au niveau de l'hétérochromatine péricentromérique avec la protéine HP1 (Protéine de l'Hétérochromatine 1) et intervient dans le remodelage de la chromatine [2]. Au niveau cellulaire, TIF1ß exerce des fonctions essentielles au cours de la spermatogenèse [3], dans le développement embryonnaire [4] et la différenciation cellulaire [5].

De manière similaire à TIF1ß, Parp-2 est associée à l'hétérochromatine péricentromérique [6], est essentielle dans les processus de différenciation cellulaire (préadipocyte en adipocyte et spermatide en spermatozoïde) [7] et joue un rôle important dans le développement embryonnaire [8].

En utilisant une approche génétique, moléculaire et cellulaire, nous avons suivi l'interaction physique et fonctionnelle de ces deux partenaires et des protéines de l'hétérochromatine HP1 dans un modèle de différenciation de cellules de carcinomes embryonnaires F9 en endoderme primitif, pariétal et viscéral. Notre travail décrit un rôle essentiel du complexe Parp-2 – TIF1ß - HP1 et de l'activité de poly(ADP-ribosyl)ation dans le contrôle épigénétique de la différenciation cellulaire.

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